CLAIMS

1. A method of quantification for cholesterol in low density lipoprotein and total cholesterol in a biological sample by a single measurement, comprising:

a first step of treating lipoproteins other than low density lipoprotein in the biological sample to generate hydrogen peroxide; and

a second step of converting the hydrogen peroxide obtained in the first step to a quinone dye and treating remaining low density lipoprotein and converting generated hydrogen peroxide to the quinone dye,

wherein the quinone dye is not formed in the first step, and cholesterol in low density lipoprotein and total cholesterol are quantified from the amount of the quinone dye formed in the second step by a single measurement.

2. The method according to claim 1, wherein reagent compositions involved in the formation of the quinone dye comprise 4-aminoantipyrine, a phenolic or anilinic hydrogen donor compound, and peroxidase; either one of 4-aminoantipyrine or the phenolic or anilinic hydrogen donor compound is added in the first step; and the reagent compositions not added in the first step is added in the second step.

3. The method according to claim 1 or 2, wherein cholesterol esterase and cholesterol oxidase are allowed to act on lipoproteins other than low density lipoprotein in the biological sample in the presence of a surfactant that acts on lipoproteins other than low density lipoprotein to generate hydrogen peroxide in the first step; and the cholesterol esterase and cholesterol oxidase are allowed to act on low density lipoprotein in the biological sample in the presence of a surfactant that acts at least on low density lipoprotein to generate hydrogen peroxide in the second step.

4. The method according to any one of claims 1 to 3, wherein in the second step, the hydrogen peroxide obtained in the first step is converted to the quinone dye; the surfactant that acts at least on low density lipoprotein is added to the measurement system; the cholesterol

esterase and cholesterol oxidase are allowed to act on low density lipoprotein remaining in the measurement system; and the generated hydrogen peroxide is measured by converting to the quinone dye.

5. The method according to any one of claims 1 to 4, wherein the amounts of cholesterol present in low density lipoprotein and total cholesterol present in the biological sample are simultaneously measured based on two values where the total amount of change in absorbance in the second step serves as a measurement value that reflects the amount of total cholesterol present and the amount of change in absorbance with respect to the amount of the hydrogen peroxide generated in the second step serves as a measurement value that reflects the amount of cholesterol present in low density lipoprotein.

6. The method according to any one of claims 1 to 5, wherein the change in absorbance in the second step shows a biphasic increase in which there are a rapid increase right after adding a second reagent and a subsequent moderate increase; and cholesterol in low density lipoprotein is quantified from the amount of the latter moderate change in absorbance.

7. The method according to any one of claims 1 to 6, wherein total cholesterol is quantified from the total amount of change in absorbance in the second step.

8. The method according to any one of claims 1 to 7, wherein analysis is performed by a single measurement under different measurement conditions using an automatic analyzer for clinical chemistry testing.

9. A method of stabilizing a liquid reagent in a method of quantification for cholesterol in low density lipoprotein and total cholesterol in a biological sample by a single measurement including a first step of adding a first reagent to treat lipoproteins other than low density lipoprotein in the biological sample to generate hydrogen peroxide and a second step of adding a second reagent to convert the hydrogen peroxide generated in the first step to a quinone dye

and to treat remaining low density lipoprotein to generate hydrogen peroxide and convert to the quinone dye, comprising:

containing either 4-aminoantipyrine or a phenolic or anilinic hydrogen donor compound that is a reagent composition involved in the formation of the quinone dye in the first reagent added in the first step; and

containing reagent compositions not contained in the first reagent among 4-aminoantipyrine, the phenolic or anilinic hydrogen donor compound, and peroxidase in the second reagent.

10. A method of stabilizing a liquid reagent in a method according to any one of claims 1 to 8, comprising:

containing either 4-aminoantipyrine or a phenolic or anilinic hydrogen donor compound that is a reagent composition involved in the formation of a quinone dye in a first reagent added in a first step; and

containing reagent compositions not contained in the first reagent among 4-aminoantipyrine, the phenolic or anilinic hydrogen donor compound, and peroxidase in a second reagent.

11. The method according to claim 9 or 10, wherein a surfactant that acts on lipoproteins other than low density lipoprotein, cholesterol esterase, and cholesterol oxidase are further contained in the first reagent; and a surfactant that acts at least on low density lipoprotein is contained in the second reagent.

12. A kit to perform a method of quantification for cholesterol in low density lipoprotein and total cholesterol in a biological sample by a single measurement including a first step of adding a first reagent to treat lipoproteins other than low density lipoprotein in the biological sample to generate hydrogen peroxide and a second step of adding a second reagent to convert the hydrogen peroxide generated in the first step to a quinone dye and to treat remaining low density lipoprotein to generate hydrogen peroxide and convert to the quinone dye, comprising:

containing either 4-aminoantipyrine or a phenolic or anilinic hydrogen donor compound that is a reagent composition involved in the formation of the quinone dye in the first reagent; and

containing reagent compositions not contained in the first reagent among 4-aminoantipyrine, the phenolic or anilinic hydrogen donor compound, and peroxidase in the second reagent.

13. The kit according to claim 12, wherein a surfactant that acts on lipoproteins other than low density lipoprotein, cholesterol esterase, and cholesterol oxidase are further contained in the first reagent; and a surfactant that acts at least on low density lipoprotein is contained in the second reagent.